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DETECTION OF RADIOFREQUENCY RADIATION - INDUCED WHOLE BODY HEATING FOLLOWING CHEMICAL IMPAIRMENT OF THERMOREGULATION

Ralph J. Smialowicz

Immunobiology Section
Experimental Biology Division
Health Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, North Carolina

I. INTRODUCTION

Heating by radiofrequency (RF) radiation at high intensities can cause biological changes by whole body hyperthermia or by altered thermal gradients within the body (Johnson and Guy, 1972). However, there have been reports of effects, such as on the reproductive, immunologic, neuroendocrine and nervous systems of experimental animals, at low intensities of RF radiation without evidence of increased body temperature (Baranski and Czerski, 1976). Over the years, there has been considerable controversy concerning the potential for RF radiation to cause various biological effects in experimental animals in the absence of detectable increases in body or tissue temperature. In many instances, the effects produced by RF radiation have been attributed to direct interactions (i.e., athermal or field-specific) unrelated to any detectable temperature change in the biological specimen. We have been particularly interested in attempting to interpret the many studies that report RF radiation-induced effects on the immune system of exposed animals in the absence of a colonic temperature increase (Smialowicz, 1979). To this end studies were undertaken to determine if subtle heating by RF radiation at low power densities ($\leq$1 mW/cm^2) might be detectable in

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animals whose thermoregulatory response was compromised.

In earlier work, Stern, *et al.* (1979) and Adair and Adams (1980), using techniques to assess behavioral and physiological thermoregulatory responses, demonstrated that animals would respond to RF radiation by selecting less infrared heat or by initiating peripheral vasodilation respectively when placed in a cool environment at power densities below 20 mW/cm^2. Putthoff, *et al.* (1977) showed increases in the core temperature of rats made hypothermic with cortisone and then exposed to RF radiation at a dose rate of approximately 40 W/kg. These results indicate that animals in which normal thermoregulatory responses are compromised by drugs or animals in which individual thermoregulatory responses are measured in cool environments may be sensitive models for the detection of subtle RF-induced heating.

To further examine this hypothesis, two agents were used to compromise the thermoregulatory response of rodents. Lipopolysaccharide (LPS) or endotoxin from Gram-negative bacteria was used in rats and 5-hydroxytryptamine (5-HT) was used in mice. LPS induces a febrile response in man and several other species; however, rats, mice and guinea pigs injected with LPS and held in a cool (22 °C) environment develop hypothermia (Feldberg and Saxena, 1975). Likewise, mice injected with 5-HT, which are held in a cool (22 °C) environment, develop a hypothermia in a matter of minutes (Dooley and Quock, 1976). Impairment of thermoregulation by endotoxin in the rat and 5-HT in the mouse in these studies allowed us to detect subtle whole body heating by RF radiation at a power density (1 mW/cm^2) heretofore considered to be nonthermogenic.

II. METHODS

The methods employed have been reported previously (Smialowicz, *et al.*, 1980, 1981a). Unless stated otherwise, experimentally naive young adult rats and mice were used once and then killed after several days of observation. Rats were injected intravenously with a single dose of Salmonella typhimuriun LPS (100 µg/kg) and mice intraperitoneally with a single dose of 5-HT creatine sulfate complex (20 mg/kg) in a volume of 0.2 ml. These agents render the animals hypothermic when they are maintained at a cool ambient temperature of 22 °C. Control animals were injected with an equal volume of pyrogen-free saline. In preliminary studies it was found that LPS was more effective in

producing hypothermia in rats than in mice where a great deal of variability in response was observed. The opposite was found with 5-HT-induced hypothermia in which mice responded better than rats.

Immediately following injection, individual rats were placed in perforated acrylic restrainers and mice were placed in perforated polycarbonate boxes. The restrained rats were positioned with their long axis oriented parallel to the H-field vector. The mice had freedom of movement in the boxes and their orientation in the field was random. Four such animals were then placed each in one of four positions of a diamond-shaped array in an environmentally controlled exposure chamber which was positioned beneath a 10-db-gain pyramidal horn antenna in the far field. The exposure chamber was confined in an anechoic chamber. The animals were then exposed to fields generated by a Varian Model PPS-2.5 AS industrial heating unit at a frequency of 2450 MHz in a continuous-wave mode (Smialowicz, *et al.*, 1980). Rats were exposed for 90 min and mice for 15 min. Species-, strain- and age-matched LPS- or 5-HT-injected animals were positioned in a similar array in a sham-irradiation chamber located outside of the anechoic chamber. A common environmental control for the RF exposure and sham-irradiation chambers permitted regulation of the temperature and humidity, unless stated otherwise, at 22 ± 0.5 °C and 50 ± 10%, with an air flow of 4.3 m^3/min.

Power density was determined using an E-field dipole probe in the absence of animals and containers at the four positions. Measurements were also made at positions with the other three occupied by water loads or animals. The specific absorption rate (SAR) was determined by twin-well calorimetry (Kinn, 1977). Briefly, an irradiated carcass and an unirradiated control of equal mass were each placed in the wells of a colorimeter and a strip chart recording was made as the calorimeter returned to equilibrium. The area under the curve of equilibrium was calculated and converted to energy absorbed by the carcass with the SAR determined from the animal's mass.

Prior to the injection of LPS or 5-HT and immediately following exposure to RF radiation, the colonic temperature of each animal was measured with a Model 520 YSI (Yellow Springs Instrument Co., Yellow Springs, Ohio) thermistor probe inserted 2.5 cm beyond the anal sphincter in mice and with a Model 401 thermistor probe inserted 8.0 cm beyond the anal sphincter in rats. Colonic temperatures were read to an accuracy of 0.1 °C from a YSI Model 46 telethermometer.

III. RESULTS

A. Detection of RF Heating in Rats

The time-dependent colonic temperature response of non-irradiated rats injected with 100 µg/kg LPS and maintained at an ambient temperature of 22 °C and relative humidity of 50% is shown in Figure 1. Under these conditions the maximal hypothermic response occurred approximately 90 minutes following injection. Colonic temperatures returned to normal after 3-4 hours. One hundred µg/kg of LPS per rat is well below the LD_{50} of 1.6 mg/kg (Smialowicz, *et al.*, 1980). Since the nadir of the hypothermic response of rats to LPS occurred at 90 minutes post-injection, subsequent experiments with RF radiation were conducted for this length of time.

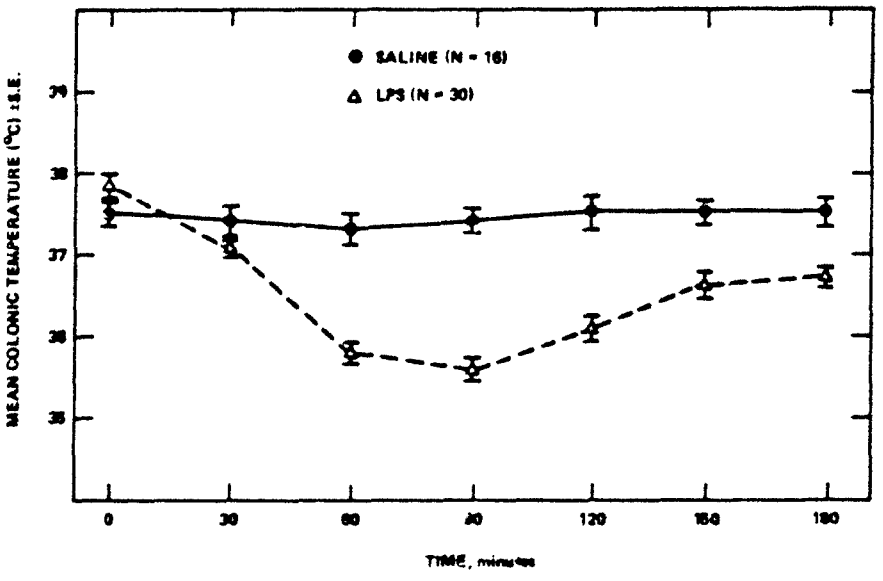

Figure 1. Time-dependent colonic temperature response to endotoxin 100 µg/kg and saline of rats held at T_a = 22 °C (Smialowicz, *et al.*, 1980. Reproduced by permission of Alan R. Liss.)

In a series of exposures to 2450 MHz radiation at different power densities (1, 5, and 10 mW/cm^2), an increase in the colonic temperature of LPS-injected rats was observed to be related to an increase in power density (Figure 2). At each power density, the mean colonic temperature was significantly greater ($P < 0.05$, Dunnett's t-test) than that of LPS-injected, sham-irradiated rats (plotted at a power density of 0 in the figure). The specific absorption rate (SAR) for rats in this study was approximately 0.2 $(W/kg)/(mW/cm^2)$. This SAR value was obtained by the method of twin-well calorimetry and has been reported by Kinn (1977) and Smialowicz, *et al.* (1979a).

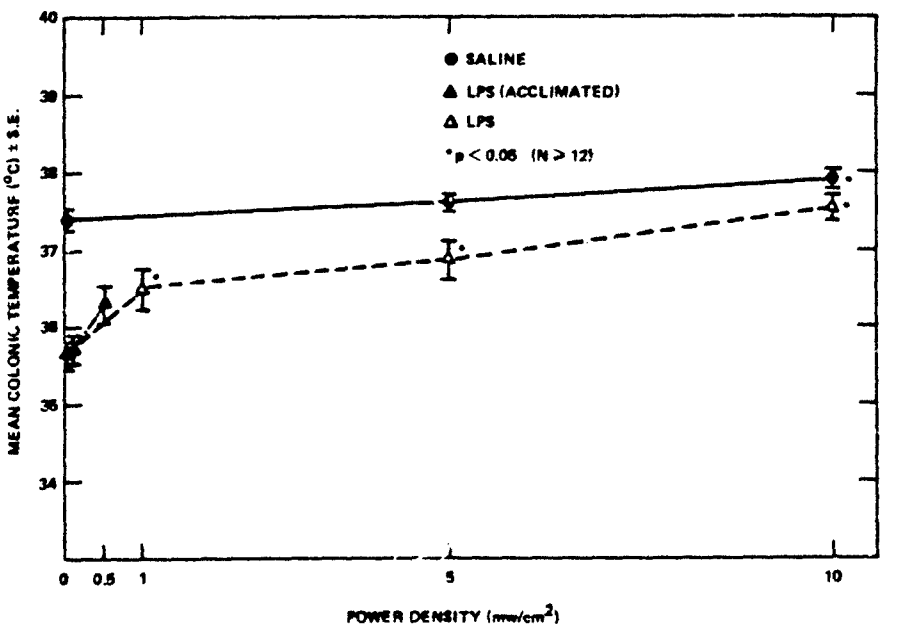

Figure 2. The effect of 90 minutes radiofrequency irradiation on endotoxin-induced hypothermia in rats. (Smialowicz, *et al.*, 1980. Reproduced by permission of Alan R. Liss.)

Rats injected with pyrogen-free saline and exposed for 90-minutes at an average power density of 5 mW/cm^2 did not show a significant increase in colonic temperature compared with saline-injected, sham-irradiated controls (Figure 2). A statistically significant difference ($P < 0.05$, Student's t-test) was observed in the colonic temperature of saline-injected rats exposed at 10 mW/cm^2, relative to saline-injected, sham-irradiated controls.

In another experiment, the effects of procedural acclimatization on the response to RF radiation was examined. Two groups of rats were acclimated to handling procedures for two weeks prior to LPS injection and RF radiation exposure. Figure 2 shows that when these rats were irradiated with 2450 MHz RF radiation at 0.5 mW/cm^2 their mean colonic temperature was 0.6 °C higher than sham-irradiated LPS-injected rats, although this difference was not significant. These results suggest that rats which are appropriately acclimated to handling and then made hypothermic with LPS may serve as an even more sensitive model than naive rats for detection of RF-induced heating.

The effect of ambient temperature alone on the magnitude of endotoxin-induced hypothermia is shown in Figure 3. In this experiment naive rats were injected with LPS (100 µg/kg), placed in restrainers and held at ambient temperatures ranging from 18 to 34 °C and at a relative humidity of 50%. Ninety minutes after LPS injection, the colonic temperature was measured. The colonic temperature of LPS-injected rats after 90 minutes depended on the ambient temperature at which the animal was held. Only when the ambient temperature exceeded 30 °C, which is at the upper limit of the thermoneutral zone for the rat, was the colonic temperature of LPS-induced hypothermic rats significantly greater than saline-injected rats held at 22 °C.

B. Detection of RF Heating in Mice

The time-dependent hypothermic response of non-irradiated BALB/C mice to 5-HT at 22 °C and 50% humidity is shown in Figure 4. The nadir of body temperature of mice treated with 5-HT occurred approximately 15 min following injection. A similar response was observed with CBA/J mice. Colonic temperature returned to normal within 90 minutes. On the basis of these data, mice were exposed to RF radiation during the first 15 min following 5-HT injection.

Table 1 shows the colonic temperature response of 5-HT injected mice exposed to 2450 MHz RF radiation at several power densities. Two strains of mice were studied, male

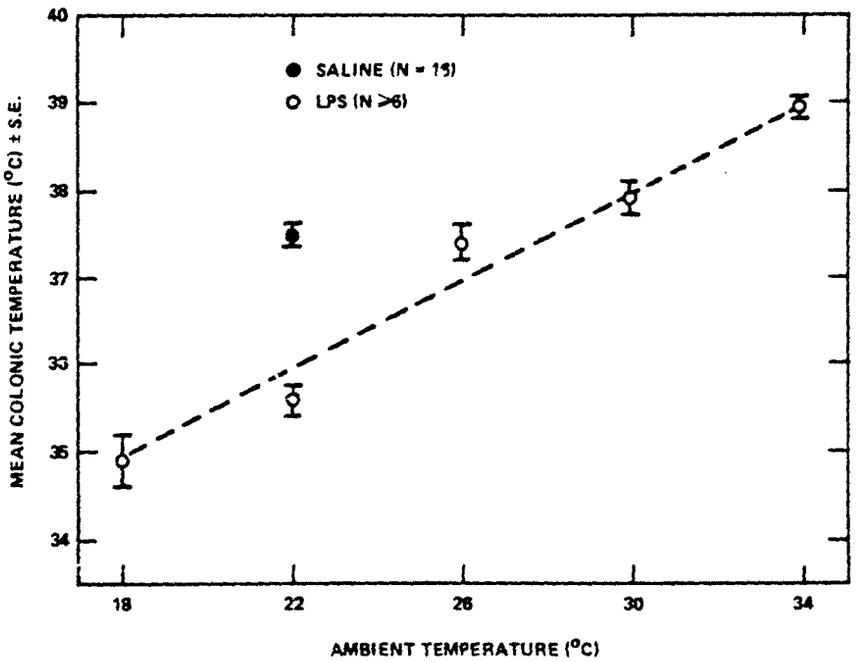

Figure 3. The effect of ambient temperature on colonic temperature of rats 90 minutes after injection of endotoxin (100 µg/kg) or saline. (Smialowicz, *et al.*, 1980. Reproduced by permission of Alan R. Liss.)

CBA/J and female BALB/C mice. These two strains were used because of our work with these mice in assessing the immunologic effects of RF radiation (Smialowicz, *et al.*, 1979b, Smialowicz, *et al.*, 1981b, and Riddle, *et al.*, 1982). The mice were maintained in an environment at 22 °C, 50% relative humidity with an air flow at 4.3 m^3/min during irradiation. Significant ($P \leq 0.05$, Student's t-test) increases in the colonic temperature of 5-HT treated BALB/C mice were observed at 10, 5 and 1 mW/cm^2 compared with 5-HT treated sham-irradiated mice. CBA/J mice irradiated at 10 and 5 mW/cm^2 also showed significantly ($P < 0.05$) higher colonic temperatures compared with their sham-irradiated controls. At 1 mW/cm^2, 5-HT treated CBA/J mice had higher colonic

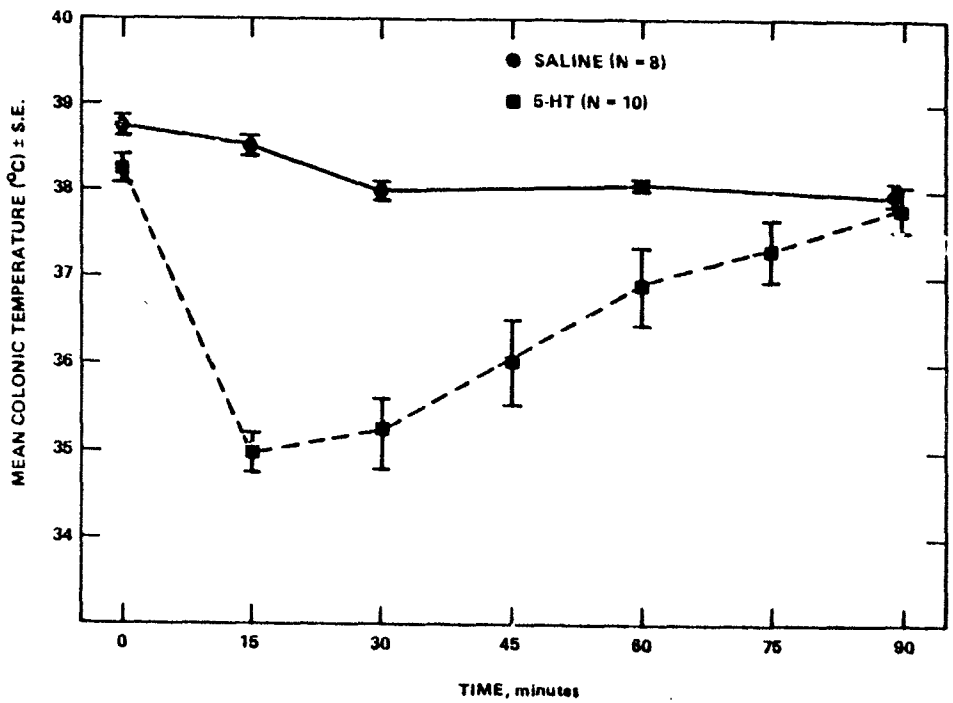

Figure 4. Time-dependent colonic temperature response of BALB/C mice held at T_a = 22 °C to 5-hydroxytryptamine (20 mg/kg) and saline (Smialowicz, et al., 1981a. Reproduced by permission of Academic Press.)

Table 1. EFFECT OF 2450 MHz RADIOFREQUENCY RADIATION ON COLONIC TEMPERATURE OF 5-HYDROXYTRYPTAMINE-INDUCED HYPOTHERMIC MICE

STRAIN	TREATMENT	POWER DENSITY (mw/cm^2)	MEAN COLONIC TEMPERATURE (°C) ± SE SHAM[a]	IRRADIATED	P
BALB/C	5-HT	1	34.3 ± 0.1	34.8 ± 0.1	< 0.05
		5	34.8 ± 0.1	35.7 ± 0.1	< 0.01
		10	34.5 ± 0.2	36.5 ± 0.2	< 0.01
	SALINE	10	38.5 ± 0.1	38.7 ± 0.1	N.S.
CBA/J	5-HT	0.5	34.2 ± 0.3	34.7 ± 0.2	N.S.
		1	34.5 ± 0.2	35.0 ± 0.2	0.051
		5	34.4 ± 0.2	35.8 ± 0.2	< 0.01
		10	34.7 ± 0.2	36.3 ± 0.3	< 0.01
	SALINE	10	37.8 ± 0.2	37.7 ± 0.1	N.S.

[a]EIGHT TO TWELVE MICE AT EACH POWER DENSITY.

Table 1. Colonic Temperature Response of Mice Injected with either Saline or 5-Hydroxytryptamine (20 mg/kg) to 2450 MHz Radiofrequency Radiation. (Smialowicz, *et al.*, 1981a. Reproduced by permission of Academic Press.)

temperatures than shams, although this difference was not significant. Saline-injected BALB/C or CBA/J mice irradiated at 10 mW/cm^2 showed no significant increase in colonic temperature compared with saline-injected sham-irradiated mice. The SAR for mice in this study was approximately 0.7 $(W/kg)/mW/cm^2$). This value was obtained by the method of twin-well calorimetry (Smialowicz, *et al.*, 1979b).

The effect of ambient temperature on the hypothermic response of 5-HT-treated CBA/J mice is shown in Figure 5. Experimentally naive mice were injected with 5-HT (20 mg/kg), placed in perforated boxes and held at ambient temperatures ranging from 18 to 34 °C and at a relative humidity of 50%. Fifteen minutes after 5-HT injection, the colonic temperature was measured. Colonic temperature increased linearly with increasing ambient temperature. Even at an ambient temperature of 34 °C, the hypothermic effect of 5-HT was still evident.

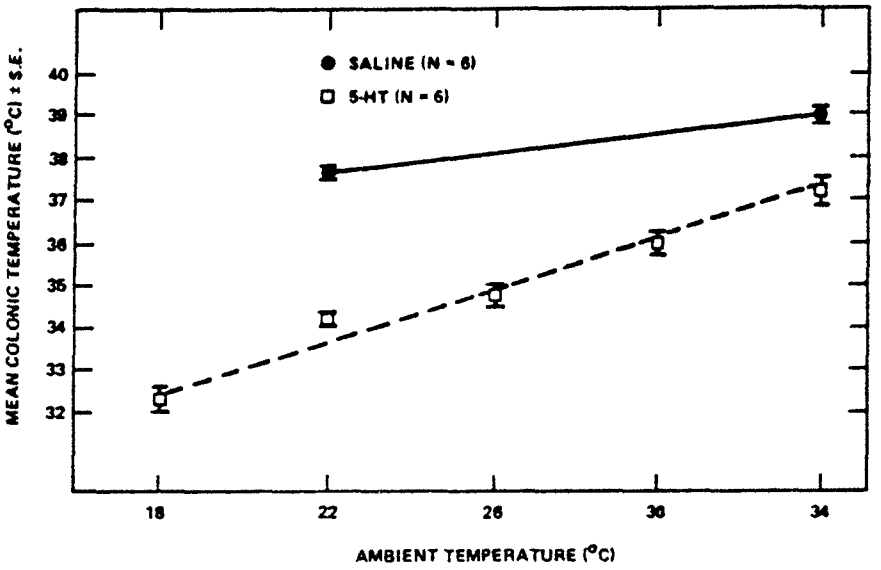

Figure 5. The effect of ambient temperature on colonic temperature of CBA/J mice 15 minutes after injection of 5-hydroxytryptamine (20 mg/kg) or saline. (Smialowicz, *et al.*, 1981a. Reproduced by permission of Academic Press.)

IV. DISCUSSION

These results demonstrate that RF heating once thought to be undetectable can be detected in rats whose normal thermoregulation was impaired by LPS and in mice whose normal thermoregulation was impaired by 5-HT at power densities heretofore considered to be nonthermogenic. Significantly higher colonic temperatures in hypothermic rats and mice compared with saline-injected animals were measured at SARs of 0.2 W/kg and 0.7 W/kg respectively. By acclimating test animals to handling procedures, the detectability of temperature increases in hypothermic rats was increased, although not significantly. These results suggest that acclimation of animals to handling procedures may reduce some of the variability observed in non-habituated LPS-injected rats and 5-HT-injected mice and increase the sensitivity of these hypothermia models for detecting subtle thermogenesis by

low-level RF radiation.

The rat, unlike other species, exhibits a decreased body temperature following intravenous injection of endotoxin. The absence of a rise in body temperature in rats following endotoxin treatment is believed to be due to a high rate of heat loss in these animals. This response may be a consequence of a reduction in insulation during shivering in the rat, which has a large body surface area to mass ratio (Bligh, 1973). Ambient temperature plays a critical role in this response to endotoxin; at temperatures above thermal neutrality, endotoxin-treated rats respond with a typical biphasic febrile response (Szekely and Szelenyi, 1979).

The systemic administration of 5-HT produces a dose-related decrease in body temperature of rats exposed to environmental temperatures below the thermoneutral point (Winter, 1971). It is believed that 5-HT hypothermia is mediated via peripheral mechanisms because systemically administered 5-HT does not cross the blood-brain barrier (Underfriend, *et al.*, 1957; Carter and Leander, 1980). The degree and direction of the change in body temperature induced by 5-HT has been shown to be a function of the ambient temperature employed and has been characterized as a poikilothermic response (Shemano and Nickerson, 1958). The exact mechanism by which 5-HT induces hypothermia is not known. It has been postulated that 5-HT acts peripherally resulting in changes in cardiovascular and/or respiratory function or may act by depressing general activity (Myers and Waller, 1977).

The colonic temperature increase in LPS-injected rats induced by RF radiation was found to be dependent on the power density at which the animals were exposed just as it was found to be dependent on ambient temperature. A similar response was observed in 5-HT-injected mice. No significant increase in the colonic temperature of rats or mice injected with saline and irradiated at 5 mW/cm^2 was observed compared with sham-irradiated saline-injected animals. These data indicate that the increase in colonic temperature in LPS-injected rats and 5-HT-injected mice exposed to RF radiation was due to the thermalizing energy of this radiation.

These results indicate that small doses of thermalizing energy from low-intensity (SAR $\leq$ 1 W/kg) RF radiation are detectable as significant increases in colonic temperature. In normal rats and mice (saline-injected), the thermalization by RF radiation at these low intensities may be compensated for by an increase in the rate of heat loss. This compensatory heat loss may then be exceeded when animals are irradiated at higher intensities, as evidence by a significant increase in the colonic temperature of saline-injected

rats exposed at 10 mW/cm^2 (Figure 2).

A re-evaluation of the literature on RF radiation-induced biological effects, particularly effects on the immune system, is indicated by these studies. More prudent assessment of claims for "nonthermal" (i.e., absence of measurable elevation of tissue or body temperature) RF-induced biological effects is needed, with consideration of the thermogenic potential of RF radiation as a cause for changes in biological responses.

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DISCLAIMER

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REFERENCES

Adair, E.R. and Adams, B.W. (1980). Microwaves induce peripheral vasodilation in squirrel monkeys. *Science* 207:1381.

Baranski, S. and Czerski, P. (1976). *Biological Effects of Microwaves*. p. 78. Dowden, Hutchinson and Ross, Stroudsburg, PA.

Bligh, J. (1973)., *Temperature Regulation in Mammals and Other Vertebrates*, p. 228. American Elsevier, New York.

Carter, R.B. and Leander, J.D. (1980). Evidence for a peripheral effect of serotonin or metabolites in 5-hydroxytryptophan-induced hypothermia. *Neurophysiol.* 19:777.

Dooley, D.J. and Quock, R.M. (1976). Tryptamine and 5-hydroxytryptamine-induced hypothermia in mice. *J. Pharm. Pharmacol.* 28:775.

Feldberg, W. and Saxena, P.N. (1975). Prostaglandins, endotoxin and lipid A on body temperature in rats. *J. Physiol. (London)* 249:601.

Johnson, C.C. and Guy, A.W. (1972). Nonionizing electromagnetic wave effects in biological materials and systems. *Proc IEEE* 60:692.

Kinn, J.B. (1977). Whole-body dosimetry of microwave radiation in small animals: The effect of body mass and exposure geometry. *Radio Sci.* 12(6S):61.

Myers, R.D. and Waller, M.B. (1977). Thermoregulation and Serotonin. In: *Serotonin in Health and Disease, Vol II. Physiological Regulation and Pharmacological Action* (W.B. Essman, ed.), Spectrum, New York.

Putthoff, D.L., Justesen, D.R., Ward, L.B., and Levinson, D.M. (1977). Drug-induced ectothermia in small mammals: The quest for a biological microwave dosimeter. *Radio Sci.* 12(6S):73.

Riddle, M.M., Smialowicz, R.J. and Rogers, R.R. (1982). Microwave radiation (2450 MHz) potentiates the lethal effect of endotoxin in mice. *Health Physics* 42:335.

Shemano, I. and Nickerson, M. (1958). Effect of ambient temperature on thermal responses to drugs. *Can. J. Biochem. Physiol.* 36:1243.

Smialowicz, R.J. (1979). Hematologic and immunologic effects of nonionizing electromagnetic radiation. *Bull. N.Y. Acad. Med.* 55:1094.

Smialowicz, R.J., Kinn, J.B. and Elder, J.A. (1979a). Perinatal exposure of rats to 2450 MHz CW microwave radiation: Effects on lymphocytes. *Radio Sci.* 14(6S):147.

Smialowicz, R.J., Riddle, M.M., Brugnolotti, P.L., Sperrazza, J.M. and Kinn, J.B. (1979b). Evaluation of lymphocyte function in mice exposed to 2450-MHz (CW) microwaves. In *Proceedings of the 1978 Symposium on Electromagnetic Fields in Biological Systems* (S.S. Stuchly, ed.) p. 122, International Microwave Power Institute, Edmonton, Canada.

Smialowicz, R.J., Compton, K.L., Riddle, M.M., Rogers, R.R., and Brugnolotti, P.L. (1980). Microwave radiation (2450 MHz) alters the endotoxin-induced hypothermic response of rats. *Bioelectromagnetics* 1:353.

Smialowicz, R.J., Riddle, M.M., Brugnolotti, P.L., Rogers, R.R., and Compton, K.L. (1981a). Detection of microwave heating in 5-hydroxytryptamine-induced hypothermic mice. *Radiat. Res.* 88:108.

Smialowicz, R.J., Brugnolotti, P.L. and Riddle, M.M. (1981b). Complement receptor positive spleen cells in microwave (2450 MHz) irradiated mice. *J. Microwave Power* 16:73.

Stern, S., Margolin, L., Weiss, B., Lu, S.-T. and Michaelson, S.M. (1979). Microwaves: Effects on thermoregulatory behavior in rats. *Science* 206:1198.

Szekely, M. and Szelenyi, Z. (1979). Endotoxin fever in rats. *Acta Physiol. Acad. Sci. Hung.* 53:265.

Underfriend, S., Weissbach, H. and Bodanski, D.F. (1957). Biochemical findings related to the action of serotonin. *Ann. N.Y. Acad. Sci.* 6:602.

Winter, J.C. (1971). Interaction of serotonin antagonists with harmaline-induced changes in operant behavior and body temperature in the rat. *Arch. Int. Pharmacodyn. Ther.* 191:120.